


## RESEARCH ARTICLE

# Structural and functional connectivity alteration patterns of the cingulate gyrus in Type 2 diabetes

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## Abstract

**Objective:** We aimed to reveal the role of structural and functional alterations of cingulate gyrus in early cognitive impairment in Type 2 diabetes mellitus (T2DM) patients. **Methods:** Fifty-six T2DM patients and 60 healthy controls (HCs) underwent a neuropsychological assessment and sagittal three-dimensional T1-weighted and resting-state functional MRI. Differences in the cortical thickness of the cingulate cortex and the functional connectivity (FC) of the nine subregions of the cingulate gyrus and the whole brain were compared between T2DM patients and HCs. Correlation analysis was performed between cortex thickness and FC and the participants' clinical/cognitive variables. **Results:** The cortical thickness of the cingulate gyrus was not significantly different between T2DM patients and HCs. However, the T2DM patients showed significantly lower FC between the pregenual ACC (pACC) and the bilateral hippocampus, significantly higher FC between the pACC and bilateral lateral prefrontal cortex (LPFC) and left precentral gyrus, and significantly lower FC between the retrosplenial cortex (RSC) and right cerebellar Crus I. The FC between the pACC and the left hippocampus was negatively correlated with the FC between the pACC and LPFC ( $r = -0.306$ ,  $p = 0.022$ ). **Interpretation:** The pACC and the RSC show dysfunctional connectivity before the appearance of structural abnormalities in T2DM patients. Abnormal FC of the pACC with the bilateral hippocampus and LPFC may imply a neural compensatory mechanism for memory function. These findings provide valuable information and new directions for possible interventions for the T2DM-related cognitive impairment.

## Introduction

Type 2 diabetes mellitus (T2DM) has become one of the most common chronic metabolic diseases in the world,<sup>1</sup> and cognitive dysfunction is being increasingly recognized as a common complication and comorbidity of T2DM.<sup>2</sup> Hyperglycemia and insulin resistance results in a neurodegenerative cascade in the central nervous system, by increasing advanced glycation end-products, oxidative stress, A $\beta$  deposition, and vascular endothelial cell damage, which reduce cognitive reserve and cause vascular cognitive impairment.<sup>3,4</sup> Cognitive impairment is a complex and progressive process,<sup>5</sup> and slight cognitive decline may be compensated for by other brain regions and

networks, allowing some cognitive units to remain relatively normal in clinical observations.<sup>6</sup> However, the onset of mild cognitive impairment (MCI) increases the probability of progression to dementia,<sup>7</sup> which can severely affect patients' self-management ability and accelerate disease progression. Therefore, exploration of the neuroimaging characteristics of early-stage cognitive impairment in T2DM patients may reveal potential clinical therapeutic targets to prevent or delay the occurrence and development of cognitive impairment in T2DM.

A large number of previous studies<sup>8–11</sup> have shown that T2DM patients without MCI have functional abnormalities in various cognitive units, including memory, executive function, and information processing speed, but the

neural mechanisms underlying these abnormalities remain unclear. Cognitive impairment is closely related to structural abnormalities and disorders of functional connectivity (FC) in key regions responsible for this function.<sup>12–14</sup> The cingulate cortex resides within the medial surface of the cerebral hemisphere and is perhaps most well known as being part of the limbic system. It seems to play a key role in multiple cognitive functions<sup>15,16</sup> and is often involved in MCI.<sup>17,18</sup> Traditionally, based on integrated neurobiological assessments, the cingulate gyrus was theoretically divided into four subregions, namely, the anterior cingulate cortex (ACC), midcingulate cortex (MCC), posterior cingulate cortex (PCC), and the retrosplenial cortex (RSC).<sup>19</sup> The ACC plays an important role in affective evaluation, memory, and attention;<sup>20–22</sup> the MCC is mainly involved in motor control and executive function<sup>23</sup>; the PCC appears to be more involved in self-reflection and autobiographical memories;<sup>24</sup> and the RSC is mainly involved in sensory, motor, and spatial cognition.<sup>25</sup> Previous studies<sup>26,27</sup> have identified the cingulate gyrus and hippocampus are the most vulnerable regions at the pre-MCI stage (subjective cognitive impairment), and some scholars<sup>28</sup> have suggested that structural and functional abnormalities in these two regions may be the neural basis for the cognitive symptoms of pre-MCI stage. Multiple studies<sup>29,30</sup> have identified hippocampal white matter disruptions and gray matter volume atrophy in T2DM patients without MCI, as well as reduced FC with multiple regions in whole brain. Although the cingulate gyrus shows obvious heterogeneity of structure and function, very few studies have comprehensively revealed the characteristics of structural and functional changes of the cingulate gyrus in T2DM patients without MCI.

Previous neuroimaging studies on T2DM have shown that patients without MCI have reduced gray matter volume in the ACC and PCC in comparison with healthy controls (HCs),<sup>31</sup> while other researchers have reported an increased density of short-range functional connections in the MCC and precuneus<sup>32</sup> and decreased nodal betweenness in the PCC.<sup>33</sup> Sun *et al.* found that reduced FC of the hippocampus with the ACC and frontal cortex may serve as an early biomarker for cognitive function-related vulnerabilities in T2DM patients.<sup>29</sup> These studies suggest that T2DM patients show abnormalities in the structure and function of the cingulate gyrus, especially the ACC and PCC, before the onset of clinically measurable cognitive impairment. Cheng *et al.* evaluated ROI-based FC to identify the dysfunctional connections between the PCC and the right superior frontal gyrus, angular gyrus, and middle temporal gyrus in T2DM patients without MCI and correlated the findings with glycosylated hemoglobin (HbA1c) levels and diabetes duration.<sup>34</sup> However, different subregions of the cingulate

cortex such as ACC, dorsal cingulate cortex, and PCC are the core brain regions of the dorsal attention network, salience network, and default mode network, respectively, and dysfunctions of these networks can lead to impairment of attention, memory and other cognitive functions.<sup>35,36</sup> Therefore, elucidating the structural and functional alterations in different subregions of the cingulate gyrus can reveal the neurological features of early cognitive impairment associated with T2DM from different perspectives.

The borders of the four subregional models of cingulate gyrus are defined on the basis of anatomical markers;<sup>16</sup> however, the FC patterns of the different subregions may not be entirely dependent on structural features, especially when a subregion includes multiple Brodmann areas. Yu *et al.* further divided the cingulate gyrus into nine subregions and found that each subregion had specific correlations and anticorrelations with cognitive, emotional, motor, and other brain networks,<sup>37</sup> providing a theoretical basis for further exploration of the FC of different subgroups of the cingulate gyrus in T2DM patients. Therefore, this study is the first to explore the FC patterns of different subregions of the cingulate gyrus and to characterize the cortical thickness changes in the cingulate gyrus in T2DM patients without MCI. We hope that the findings will reveal the role of the cingulate gyrus in early cognitive impairment in T2DM from both functional and structural aspects. We also speculate that the structure and FC of the ACC and PCC, which are closely related to cognitive function, show abnormal changes in T2DM, and that these changes are related to neuropsychological scores.

## Materials and Methods

### Participants

The study population included 59 patients with T2DM who presented at the Endocrinology Department of Shaanxi Provincial People's Hospital from May 2018 to October 2022 and 62 HCs who underwent evaluations at the health examination center of our hospital. All participants were aged 45–70 years, right-handed, and had at least 6 years of education. The Mini-Mental State Examination (MMSE) score  $\geq 27$ ; and the Montreal Cognitive Assessment (MoCA) score  $\geq 26$ . The inclusion criteria for the HC group were as follows: no symptoms of T2DM; fasting blood glucose (FBG) concentration of  $<7.0$  mmol/L; glycated hemoglobin (HbA1c) of  $<6.0\%$ . T2DM was diagnosed in accordance with the 2014 American Diabetes Association diagnostic criteria, and patients with T2DM were receiving stable therapy (diet, oral medications, and/or insulin).

Exclusion criteria for all subjects (T2DM and HC) were as follows: severe claustrophobia or contraindications to MRI; Parkinson's disease, alcoholism, major depression, epilepsy, brain injury, or other neurological or psychiatric disorders; or any other systemic disease. In addition, T2DM patients with a history of hypoglycemia (blood glucose concentration  $<3.9$  mmol/L) or hyperglycemia (blood glucose concentration  $>33.3$  mmol/L) will also be excluded.

On the day of the scan, participants arrived at the department for MRI scans after dinner at 6:30–7:00 PM and underwent blood glucose control in accordance with their doctor's instructions. The MRI scans were obtained after an interval of approximately 30 min, during which the participants underwent a structured clinical interview and a series of psychological tests. The test procedure and scan time were the same for HCs and patients with T2DM. However, to ensure relatively stable blood glucose levels when the participants completed the examination, only one participant underwent scans each day. All participants remained awake during the scan and did not experience discomfort. This study was approved by the ethics committee of Shaanxi Provincial People's Hospital, and all participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations of the Declaration of Helsinki.

### Clinical data collection and neuropsychological testing

The participants' medical history and clinical data were obtained from medical records and questionnaires, and included data for blood pressure, height, weight, and body mass index (BMI). The HbA1c, fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels were measured by standard laboratory tests. The participants' mental status and cognitive condition were evaluated using a series of neuropsychological tests, including the MMSE and MoCA to assess general cognitive function; the Color Trails Test part 1 and part 2 (CTT-1 and CTT-2) to test attention and executive functions; the Clock-Drawing Test (CDT) to evaluate visuospatial skills; and the total immediate recall and delayed recall scores in the Rey Auditory Verbal Learning Test (RAVLT) for assessing memory function. Processing speed was evaluated using the Symbol Digit Modalities Test (SDMT). All neuropsychological tests were administered by a psychiatrist with at least 5 years of experience. All participants completed the full battery of neuropsychological tests, except 12 HCs who did not participate in the RAVLT and SDMT tests.

Lacunar infarcts and white matter hyperintensity (WMH) on fluid-attenuated inversion recovery (FLAIR)

images were quantified using an age-related white matter change scale<sup>38</sup> with a single-blind method, and participants with ratings  $>2$  were excluded.

### Image acquisition

MRI scans were acquired using a 3.0T MR scanner (Ingenia, Philips Healthcare, The Netherlands) with a 16-channel phased-array head coil. Routine T2-weighted and FLAIR sequences were used to exclude visible brain lesions. Sagittal 3-dimensional T1-weighted imaging (T1WI) was performed using a fast spoiled gradient echo sequence; repetition time (TR), echo time (TE), flip angle (FA), field of view (FOV), matrix, slice thickness, and the number of slices were 7.5 ms, 3.5 ms,  $8^\circ$ ,  $250\text{ mm} \times 250\text{ mm}$ ,  $256 \times 256$ , 0.55 mm (no gap), and 328, respectively. Resting-state functional MRI (rs-fMRI) images were acquired using a gradient echo planar sequence; TR, TE, FA, FOV, matrix, the number of slices, slice thickness, and the number of volumes acquired in each scan were 2000 ms, 30 ms,  $90^\circ$ ,  $230\text{ mm} \times 230\text{ mm}$ ,  $128 \times 128$ , 34, 4 mm (no gap), and 200, respectively, with axial interleaved acquisition. The participants were instructed to close their eyes and remain awake throughout the scan.

### Processing of structural MRI

T1-weighted structural images were processed using the Computational Anatomy Toolbox 12 (CAT12: <http://www.neuro.uni-jena.de/cat/>) software for SPM12 in MATLAB. The cortical thickness estimation in CAT12 is performed automatically using a projection-based thickness method<sup>39</sup> with the following steps: automatic segmentation of the images to gray matter, white matter, and cerebrospinal fluid; affine registration to a Montreal Neurological Institute (MNI) template space; and non-linear deformation. Then, thickness estimation was performed automatically in CAT12, which included calculating the white matter distance, projecting the local maxima, topological correction, spherical mapping, and spherical registration. Finally, the structural images were smoothed with a 15-mm FWHM Gaussian kernel.

### Preprocessing of resting-state fMRI data

The DPABI (<http://rfmri.org/dpabi/>) software package was used to preprocess rs-fMRI data.<sup>40</sup> After discarding the first 10 time points to ensure a stable magnetic field, the remaining 190 time points were subjected to slice-timing and realignment of head motion correction. Participants showing head motion  $>1.5$  mm and/or translation  $>1.5^\circ$  rotations were excluded, and the effect of high-head motion was reduced using the scrubbing method.<sup>41</sup>

The framewise displacement (FD) was calculated, and the FD threshold for bad volumes was set at 0.2 mm. Bad volumes with FD >0.2 mm along with one forward and two back volumes of the bad volumes were scrubbed. Next, each bad volume was modeled as a regressor in the model regression,<sup>42</sup> and the images were normalized to the standard MNI space with a resampling voxel size of 3 mm × 3 mm × 3 mm. We also regressed out 24 head motion parameters, the linear trend signal, and the cerebrospinal fluid and white matter signals. Finally, the effect of physiological noise was regressed using a temporal-band filter (0.01–0.08 Hz).

### Functional connectivity analysis

The cingulate cortex was divided into nine regions of interest (ROIs) according to a previous study,<sup>37</sup> and each ROI was manually defined on the ch2bet structural template with the MRIcron software ([www.mricron.com](http://www.mricron.com)). ROI 1 represented the subgenual ACC (Area 25); ROI 2 and ROI 3 represented the posterior (Area 24) and anterior (Area 32) parts of the pregenual ACC (pACC), respectively; ROI 4 and ROI 5 represented the inferior (Area 24) and superior (Area 32) parts of the anterior MCC, respectively; ROI 6 represented the posterior MCC (Area 24); ROI 7 represented the dorsal PCC (Areas 23, 31); ROI 8 represents the ventral PCC (Areas 23, 31); and ROI 9 represented the RSC (Areas 29 and 30). Then, all ROIs (Fig. 1) were extracted for voxel-wise FC analyses.

For each participant, the mean time series were extracted for each ROI, and correlations were calculated between each ROI and every other voxel within the brain

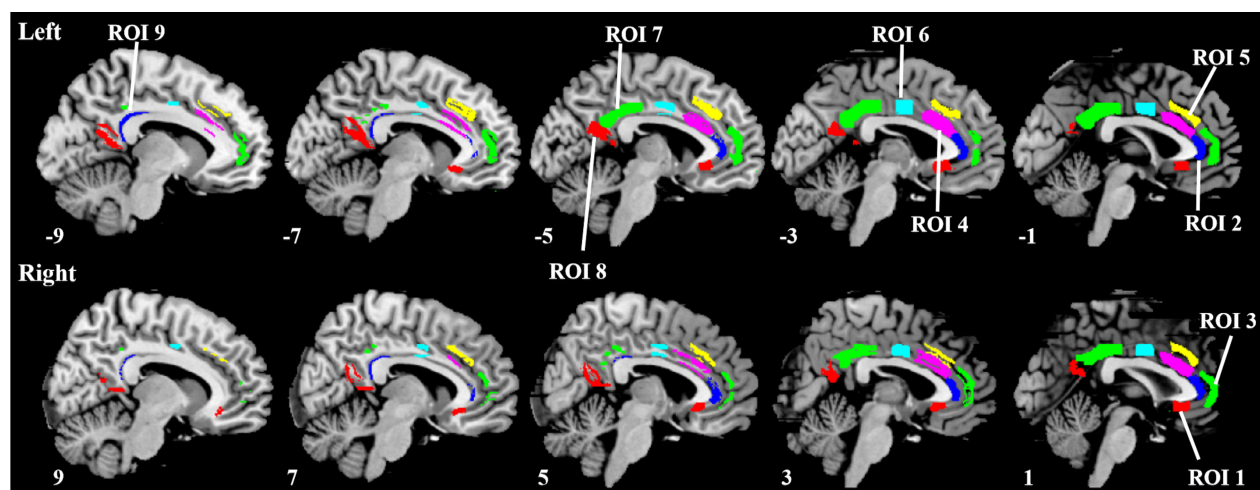
to obtain FC maps. These FC maps were converted to z-score maps using Fisher's z transformation to improve normality. Then a two-sample *t*-test was performed to investigate the regions showing significant differences in zFC between two groups, with BMI values as covariates (GRF-corrected  $p < 0.001$ , cluster level  $p < 0.05$ ).

To investigate the correlations between zFCs of the cingulate subregions with significant group differences and clinical variables, we extracted the average zFC values and performed partial correlations (Bonferroni-corrected,  $p < 0.05$ ) between the zFC values and clinical variables, with BMI values as covariates.

### Statistical analyses

SPSS Statistics version 24 (IBM Corporation, Armonk, NY, United States) was used to perform statistical analyses. Group differences in normally distributed variables were detected using independent-sample *t*-tests, while non-normally distributed variables were evaluated using the Mann–Whitney *U*-test. Group differences in sex were assessed using the chi-squared ( $\chi^2$ ) test. *p*-values less than 0.05 indicated statistical significance.

Statistical analysis of the cortical thickness was performed using two-sample *t*-tests with BMI values as covariates. The threshold was set at a significance level of  $p < 0.05$  and was family-wise error (FWE)-corrected for multiple comparisons. For zFCs of the cingulate subregions, two-sample *t*-test were performed to investigate the regions with significant differences between two groups, with BMI values as covariates (voxel level  $p < 0.001$ , cluster level  $p < 0.05$ , GRF-corrected).



**Figure 1.** ROIs of the cingulate subregions. ROIs of each side of the cingulate cortex are shown on 10 sagittal anatomical images of the ch2bet template. The positive values (1, 3, 5, 7, 9) of the x-axis coordinates represent the right hemisphere, while the negative values (−1, −3, −5, −7, −9) denote the left hemisphere. Each color represents a ROI, which is labeled in the figure.

To investigate the correlations between zFCs of the cingulate subregions with significant group differences and clinical/cognitive variables, we extracted the average zFC values and performed partial correlations (Bonferroni-corrected,  $p < 0.05$ ) between the zFC values and clinical/cognitive variables, with BMI values as covariates.

## Results

### Clinical and neuropsychological data

Five participants were excluded from the final statistical analysis; one T2DM patient was excluded for excessive motion, and four participants (two with T2DM and two HCs) were excluded for a WMH score  $>2$ . A total of 56 patients with T2DM and 60 HCs were enrolled in the study. The demographic, clinical, and neuropsychological data of the two groups are presented in Table 1. The two groups showed no significant differences in age, sex, education level, blood pressure, TG concentration, TC concentration, LDL-C concentration, CTT-2, MMSE, MoCA, CDT, RAVLT immediate/delayed, and SDMT score ( $p > 0.05$ ). However, the BMI and CTT-1 score were

higher in the T2DM group than in the control group (all  $p < 0.05$ ). In addition, the T2DM group showed higher FBG and HbA1c levels than the control group (all  $p < 0.001$ ). The T2DM group included 27 patients with no complications and 29 patients with complications such as nephropathy, peripheral neuropathy, and retinopathy (Table S1).

### Between-group differences in cingulate cortex thickness and subregion FC

After controlling for BMI ( $p < 0.05$ ), the cortical thickness of the cingulate gyrus was not significantly different between T2DM patients and HCs. However, in comparison with HCs, patients with T2DM showed significantly reduced FC between the pACC and the bilateral hippocampus, but had significantly higher FC between the pACC and bilateral lateral prefrontal cortex (LPFC) and left precentral gyrus ( $p < 0.05$ ) (Fig. 2A, Table 2). In addition, the T2DM group showed significantly lower FC between the RSC and right cerebellar Crus I ( $p < 0.05$ ) (Fig. 2B, Table 2). Furthermore, to confirm that the increased FC of the pACC and left precentral gyrus in the

**Table 1.** Demographic, clinical, and neuropsychological characteristics of the participants.

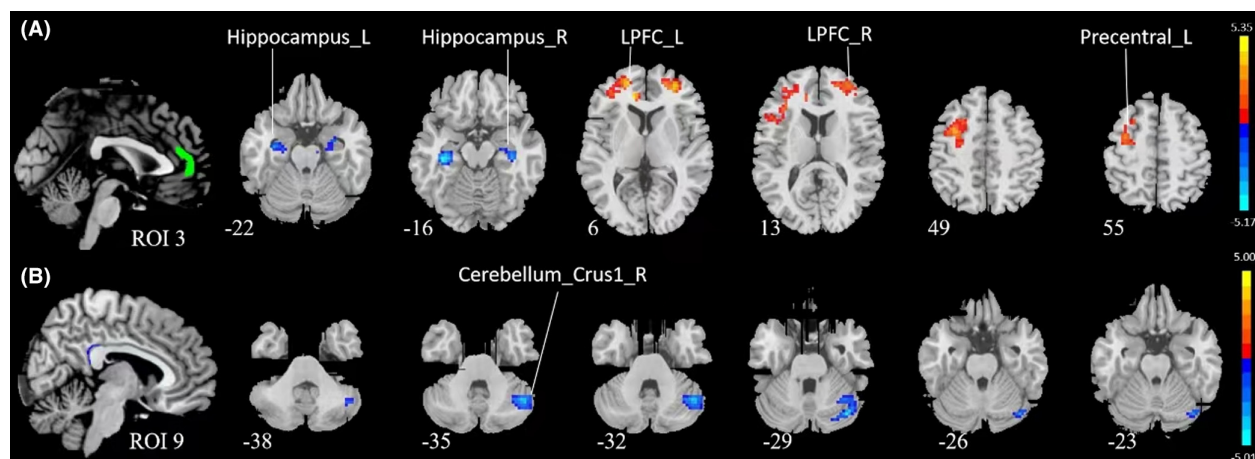
Variable	T2DM patients ( $n = 56$ )	HC ( $n = 60$ )	$t/\chi^2$ value	$p$ -value
Sex (male/female)	42/14	38/22	1.842	0.175 <sup>#</sup>
Age (years)	52.89 $\pm$ 10.28	54.35 $\pm$ 6.05	-0.937	0.351
Education (years)	14.32 $\pm$ 2.24	13.81 $\pm$ 3.62	0.895	0.373
Duration (years)	7.37 $\pm$ 5.69	—	—	0
Systolic BP (mmHg)	125.92 $\pm$ 16.93	123.71 $\pm$ 11.51	0.827	0.410
Diastolic BP (mmHg)	79.42 $\pm$ 10.20	79.53 $\pm$ 8.91	-0.059	0.953
BMI (kg/m <sup>2</sup> )	27.63 $\pm$ 9.00	25.75 $\pm$ 3.05	2.546	0.012*
FBG (mmol/L)	8.32 $\pm$ 3.13	4.16 $\pm$ 2.06	8.504	<0.001*
HbA1c (%)	8.07 $\pm$ 2.04	5.29 $\pm$ 1.32	8.755	<0.001*
TG (mmol/L)	2.43 $\pm$ 1.63	1.80 $\pm$ 1.08	1.633	0.105
TC (mmol/L)	4.33 $\pm$ 1.44	4.30 $\pm$ 1.17	0.123	0.903
LDL-C (mmol/L)	2.62 $\pm$ 0.90	2.65 $\pm$ 0.82	-0.191	0.849
CTT-1	74.98 $\pm$ 25.54	66.15 $\pm$ 16.96	2.207	0.029*
CTT-2	151.89 $\pm$ 48.47	136.29 $\pm$ 55.83	1.632	0.105
MMSE	29.05 $\pm$ 0.94	28.86 $\pm$ 1.52	0.788	0.432
MoCA	27.41 $\pm$ 1.35	27.60 $\pm$ 1.21	-0.828	0.409
CDT	28.16 $\pm$ 2.98	27.95 $\pm$ 2.14	0.440	0.661
RAVLT immediate	43.34 $\pm$ 6.70	44.85 $\pm$ 8.63	-0.788	0.433
RAVLT delay	8.38 $\pm$ 24.83	9.14 $\pm$ 3.07	-1.064	0.290
SDMT	45.72 $\pm$ 12.97	48.19 $\pm$ 7.01	-0.907	0.367

Normally distributed variables are presented as mean  $\pm$  standard deviation, and variables that are non-normally distributed are presented as median (minimum, maximum).

BMI, body mass index; CDT, Clock-Drawing Test; CTT-1, Color Trails Test part 1; CTT-2, Color Trails Test part 2; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HC, healthy control; LDL-C, low-density lipoprotein cholesterol; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; RAVLT: Rey Auditory Verbal Learning Test; SDMT: Symbol Digit Modalities Test; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride.

<sup>#</sup> $p$  for the  $\chi^2$  test.

\* $p < 0.05$ .



**Figure 2.** Resting-state FC differences in the cingulate subgroups between the T2DM and HC groups (voxel level,  $p < 0.001$ ; cluster level,  $p < 0.05$ ; GRF-corrected). (A) Brain regions with differential FCs of the pACC in patients with T2DM and HCs. (B) Reduced FC of the RSC in T2DM patients in comparison with HCs. Warm (cold) color indicates significantly increased (decreased) FC.

**Table 2.** Abnormal FC of the cingulate subregions in the patients with T2DM compared to the HC group.

Brain regions	Peak MNI			Cluster size	BA	T-value
	X	Y	Z			
ROI 3						
Hippocampus_R	36	-24	-15	54	20	-4.5344
Hippocampus_L	-33	-24	15	78	20	-5.4763
LPFC_L	-12	39	6	294	10/ 46	5.2996
LPFC_R	33	51	9	102	10	4.8333
Precentral_L	-33	6	48	135	6	4.4569
ROI 9						
Cerebellum_Crus1_R	45	-57	-30	158	-	-4.9117

BA, Brodmann area; L, left; LPFC, lateral prefrontal cortex; MNI, Montreal Neurological Institute; R, right; ROI, region of interest.

T2DM group may be caused by diabetic peripheral neuropathy (DPN) in this study, we compared the difference between the FC of the pACC and the whole brain in T2DM patients with and without DPN. The results showed that in comparison with T2DM patients without DPN, those with DPN showed increased FC between PACC and the left precentral gyrus/middle frontal gyrus (BA6/9) and the bilateral supplementary motor area/left superior frontal gyrus (BA6/8) (Table S2 and Fig. S1). No other regions with abnormal FC were observed in the groups.

### Correlation between the FC and clinical/cognitive variables

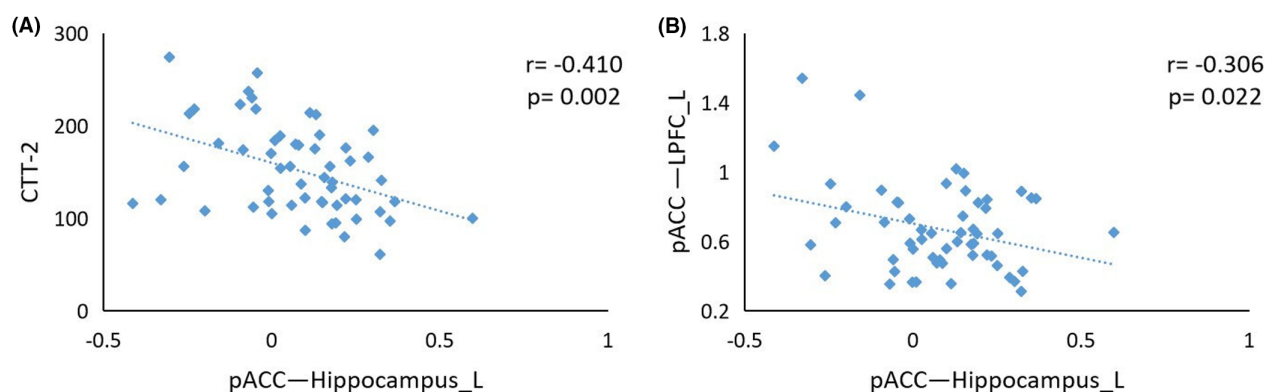
After controlling for BMI ( $p < 0.05$ ), the FC between the pACC and the left hippocampus was negatively correlated with the CTT-2 score ( $r = -0.410$ ,  $p = 0.002$ ) in T2DM

patients, after Bonferroni correction for  $p$  (Fig. 3A). The FC showed no significant correlations with other clinical/cognitive variables in both T2DM and HC groups. Moreover, the FC between the pACC and the left hippocampus was negatively correlated with the FC between the pACC and left LPFC ( $r = -0.306$ ,  $p = 0.022$ ) (Fig. 3B) in T2DM patients, whereas this correlation did not exist in HCs.

### Discussion

This study investigated the change characteristics in cingulate cortex thickness and the patterns of altered FC in different subregions of the cingulate gyrus in T2DM patients. The results revealed that T2DM patients without clinically measurable cognitive impairment still had disrupted FC between subregions of the cingulate gyrus and multiple brain regions before showing structural alterations. Although structural changes underlie functional brain abnormalities, functional abnormalities often precede structural damage, and brain structural changes may not be evident in the early stages of functional impairment in several neurodegenerative diseases.<sup>43,44</sup> Notably, this study found that the FC values of the pregenual ACC and the left hippocampus were negatively correlated with the FC values of the pregenual ACC and the left LPFC, indicating the presence of effective compensatory mechanisms to counteract the early slight cognitive impairment in T2DM patients without MCI.

Long-term memory functions are categorized into encoding, consolidation and retrieval, which coordinate with each other to achieve long-term memory. ROI 3 (pACC, Area 32) plays a crucial role in memory consolidation; it links neocortical representational areas in long-



**Figure 3.** (A) Significant negative correlation between the CTT-2 score and the FC between the pACC and the left hippocampus in T2DM patients ( $r = -0.410$ ,  $p = 0.002$ ). (B) The FC between the pACC and the left hippocampus was negatively correlated with the FC between the pACC and left LPFC ( $r = -0.306$ ,  $p = 0.022$ ).

term memory and suppresses irrelevant representations in the limbic system.<sup>45</sup> The hippocampus has long been established as playing important roles in memory encoding and retrieval,<sup>46</sup> and the ACC projection has been suggested to have direct top-down control over the hippocampal memory processing.<sup>47</sup> A recent animal experiment showed that synaptic plasticity in the ACC occurred 1 day after learning, which most likely reflects a neural mechanism by which memory is transferred from the hippocampus to the ACC to achieve memory consolidation.<sup>48</sup> Therefore, we speculated that the reduced FC between the pACC and hippocampus may reflect the impaired pACC-hippocampal memory pathway in patients with T2DM. In addition, this study also found that reduced FC of the pregenual ACC and left hippocampus was negatively correlated with the CTT-2 score, suggesting that disturbed FC between the pregenual ACC and left hippocampus is associated with worse executive function in T2DM patients. Executive function is closely related to memory,<sup>49</sup> and executive function can predict the validity of subjective memory complaints and working memory function.<sup>50,51</sup> This study showed a trend of significant differences in the CTT-2 scores between T2DM patients and HCs and imply a slight alteration of memory function in T2DM patients.

Although core brain regions play key roles in cognitive function, cognitive processes actually require the cooperation of multiple brain regions.<sup>52–54</sup> In meta-analyses of episodic memory retrieval, the LPFC consistently emerged as a core locus of activation in addition to the hippocampus.<sup>55,56</sup> The LPFC is involved in narrowing the retrieval scope and supporting accurate situational retrieval,<sup>57</sup> and participants show increased prefrontal activation<sup>58,59</sup> or exhibit strengthened prefrontal FC to memory retrieval networks<sup>60</sup> to maintain better working memory performance, suggesting a compensatory function

of the LPFC in working memory. Although the lack of RAVLT scores in some HC patients in this study may have affected the statistical power, T2DM patients did not show a trend of memory impairment based on the current data. Therefore, we speculated that the increased FC between the pregenual ACC and bilateral LPFC may be a compensatory mechanism for the damaged memory pathways. Interestingly, our study showed that the decreased FC of the pACC and left hippocampus were negatively correlated with the increased FC of the pACC and left LPFC, further confirming our speculation. Deng *et al.*<sup>61</sup> found that the prefrontal cortex (PFC) reconfiguration increases in older adults and tracked reconfiguration reductions in the medial temporal lobe (MTL), with a “seesaw” relationship between the MTL and PFC suggesting that strengthened PFC connectivity may compensate for MTL deficits. This study implies that this “seesaw” relationship may also exists in T2DM patients without MCI, and that the LPFC may compensate for poor hippocampal function in T2DM patients. Although several previous neuroimaging studies<sup>62–64</sup> have suggested a compensatory mechanism in the brain function of T2DM patients without MCI, none of them can provide strong evidence. Our results may provide some imaging basis for the compensatory mechanisms in the brain network in T2DM, which have been difficult to prove for a long time.

In addition to participating in cognitive functions such as memory and emotion, the pACC also interacts with the sensorimotor network.<sup>37</sup> The precentral gyrus, a classical sensorimotor area, plays an important role in the processing of sensory and motor signals. Accordingly, we hypothesized that increased FC of the pACC with the precentral gyrus in patients with T2DM may be related to an abnormal sensorimotor network. In patients with DPN, the precentral gyrus shows reduced gray matter volume and abnormal neuronal activity,<sup>65,66</sup> indicating a close association between DPN and abnormal central

sensorimotor function. In this study, in comparison with patients without DPN, those with DPN showed increased FC between the pACC and multiple sensorimotor regions (including the precentral gyrus), which partly validates our speculation. Rocca *et al.* found that patients with peripheral neuropathy showed decreased FC within the sensorimotor network but increased FC with multiple other sensory and cognitive network regions, which may reflect the mechanism of central adaptation to peripheral injury.<sup>67</sup> A previous study<sup>68</sup> also reported that enhanced FC between the pACC and the precentral gyrus was associated with neurological symptoms in patients with spinal cervical spondylosis with limb sensory deficits, suggesting that this may be a compensatory mechanism designed to preserve neurological function caused by spinal cord injury downstream within the motor network. However, whether the enhanced FC in this study is also a compensatory mechanism will require further analysis and verification by collecting detailed clinical data and neuroimaging findings at a later stage.

The latest view suggest that since the RSC anatomy and dynamics are more consistent with roles in multiple sensorimotor and cognitive processes, including sensory, motor, memory, and visuospatial function, than with any isolated function, this region can encode conjunctions for these functions and provide a scaffold for intelligent actions, such as navigation and perspective-taking.<sup>25</sup> The cerebellar Crus I is a part of the posterior cerebellum, which facilitates such cognitive functions as language, memory, spatial processing, and executive control and serves as a crucial auxiliary region allowing the cerebrum to perform higher cognitive functions.<sup>69,70</sup> The RSC and cerebellar Crus I are functionally complex and diverse but not central to a particular cognitive function, so we hypothesize that reduced FC between these regions may only suggest a widespread but slight cognitive abnormality in patients with T2DM. The posterior cerebellar regions may be sensitive to T2DM-related damage<sup>71,72</sup>; moreover, T2DM often presents with extensive cerebellar-cerebral circuit impairment,<sup>70,73</sup> and our findings further enrich this theory. In addition, our findings showed significantly higher CTT-1 scores in T2DM patients than in HCs, indicating that these patients have impaired attention. Although previous studies showed dysfunctional connections in the ventral attention network even in pre-diabetic patients,<sup>74</sup> the present study did not find abnormalities in the attention network, which may be related to the differences in the study objectives and research methods.

## Limitations

This study had several notable limitations. First, although the missing RAVLT and SDMT scale scores for some HCs

prevented an accurate assessment of the presence of impairments in memory and attention function in these T2DM patients. However, since all T2DM patients completed all cognitive scales, the missing data did not greatly affect the analyses of the correlation of neuroimaging results with the neuropsychological scales. Second, T2DM patients with complications were not excluded, and the difference in FC between pACC and precentral gyrus could be attributed to the inclusion of 27 patients with DPN, which introduced some bias in our results. However, the small number of patients with diabetic nephropathy and retinopathy limited the impact of these conditions on the outcomes. Third, the differences in the treatment regimens and drugs for T2DM among patients may have introduced some bias; however, such differences are difficult to avoid.

## Conclusion

The cingulate gyrus shows dysfunctional connectivity in multiple subregions before the appearance of structural abnormalities in T2DM patients without MCI, and the abnormal FC of the pACC with the bilateral hippocampus and LPFC may involve a neural compensatory mechanism for memory function. These findings reveal the role of dysfunctional connectivity of the cingulate gyrus in early cognitive impairment in T2DM and provide valuable information and new directions for possible interventions for the cognitive impairment associated with T2DM.

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## Conflict of Interest

The authors report no competing interests.

## Author Contributions

D.Z. drafted the manuscript and designed the experiment. Y.H. performed the statistical analysis. S.L. contributed to performing the experiments and revised the manuscript. J.G., W.L. (Weirui Liu), and W.L. (Wanting Liu) collected the data. K.A. provided technical support. X.L. and X.Z. revised the manuscript. All authors have read and agreed to the submit the final version of the manuscript.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

### Appendix S1